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The Effects of Hypertonic Dextrose Injection on Connective Tissue and Nerve Conduction through the Rabbit Carpal Tunnel

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Abstract

Objective—To investigate the effects of hypertonic dextrose injection on the subsynovial connective tissue (SSCT) in a rabbit model. We hypothesized that dextrose injection would induce proliferation of the SSCT, hinder median nerve conduction, and alter SSCT mechanical properties similar to what is observed in patients with carpal tunnel syndrome (CTS).

Design—Randomized, controlled prospective study.

Setting—Not applicable.

Participants—New Zealand white rabbits (N=28) weighing 4.0 to 4.5kg.

Intervention—One fore paw was randomly injected with 0.1ml of 10% dextrose solution. The contralateral paw was injected with a similar amount of 0.9% saline solution as a control. Animals were sacrificed at 12 weeks after injection.

Main Outcome Measures—Animals were evaluated by electrophysiology (EP), mechanical testing, and histology. EP was evaluated by distal motor latency and amplitude. Shear force was evaluated when the middle digit flexor digitorum superficialis tendon was pulled out from the carpal tunnel. The ultimate tensile load and the energy absorption were also measured. Tissue for histology was evaluated qualitatively.

Results—EP demonstrated significant prolongation of distal motor latency. The energy absorption and stiffness were also significantly increased in the dextrose group. Histologically, the dextrose group showed thickening of the collagen bundles and vascular proliferation within the SSCT compared to the saline group.

Conclusions—These results are consistent with the findings in CTS patients and suggest that hypertonic dextrose injection has the potential to create a novel animal model in which to study the evolution of CTS.

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Keywords

Carpal tunnel syndrome; Models; animal; Rehabilitation

Carpal tunnel syndrome is a common, chronic, pressure induced neuropathy of the median nerve. The prevalence of CTS has been reported to be 2% to 4% in the adult population,^{1,2} and the incidence is increasing.^{3,4} In the United States, the crude incidence of CTS in the population has been estimated at 99 per 100,000 person years, with an age-adjusted incidence of 149 for women and 52 for men,⁵ or even higher in more recent reports.⁶ Although an increased carpal tunnel pressure is observed in CTS patients compared with healthy control subjects,⁷⁻⁹ most cases of CTS do not have an identifiable etiology.

Within the carpal tunnel, there are 2 bursae. The radial bursa envelopes the flexor pollicis longus, and the ulnar bursa surrounds the other flexor tendons and the median nerve. The inner lining of the bursa is the visceral synovium, which cover the flexor tendons and median nerve. Beneath the visceral synovium, there is a mesh-work of areolar connective tissue,¹⁰ which connects the visceral synovium, tendons, nerve, and synovium, which is called the SSCT.^{11, 12} The SSCT consists of multiple layers of collagenous fibers, in which the blood and lymphatic vessels are richly represented.^{11,12} During tendon motion, this tissue serves as a sliding unit to reduce friction and protect the vascular supply to tendons and synovium.¹⁰ The most characteristic histopathologic findings in idiopathic CTS patients are noninflammatory fibrosis and thickening of the SSCT.¹¹⁻¹⁴ Lluch¹⁵ suggested that SSCT fibrosis might be caused by repetitive shear stress, and be a cause of the neuropathy, but the role of the SSCT in the pathogenesis of CTS has not, to date, been studied in detail.

To clarify the pathogenesis of CTS, several investigators have developed animal models. However, these approaches have focused on the pathology of the acute nerve compression, rather than the etiology or the pathogenesis of the compression itself. In these models an acute pressure effect has been created by direct compression,¹⁶⁻¹⁹ infusion of a saline solution,¹⁶ the use of tubing with various internal diameters,^{17,18} or surgically tightening the flexor retinaculum.¹⁵ Although demyelination of the median nerve has been observed in those experiments, these approaches model the effects of pressure on nerve, and not what might induce the pressure in the first place. Thus, while being good models of nerve pathology in response to pressure, they are not good models of CTS per se.

Prolotherapy, also known as regenerative injection therapy, is an alternative medical treatment to cure various musculoskeletal problems.¹⁹ It is defined as the treatment involving the injection of growth factors or growth factor production stimulants to promote regeneration of normal cells and tissue.^{20,21} A large body of supportive research has examined different types of proliferation stimulants. The most common stimulant used clinically is hypertonic dextrose.²² Although the therapeutic mechanisms of action for prolotherapy are not clearly understood, experimental data support the effectiveness of common prolotherapy agents for inducing cellular proliferation and vascular changes.²³ It has been shown that 10% dextrose is an effective and clinically tolerable concentration, and so the most acceptable to patients.²⁴ In addition, 10% dextrose has been shown to create a noninflammatory reparative effect in a rabbit model.²⁵ This suggests that 10% dextrose may be useful to create a similar noninflammatory regenerative change in a rabbit carpal tunnel model as well.

In a pilot study, a single injection of 10% dextrose did indeed induce progressive fibrosis in the SSCT in a rabbit model.²⁶ Based on this preliminary data, we designed a study to investigate more completely the effects of hypertonic dextrose injection on the SSCT in the rabbit model, specifically to determine if the single injection of hypertonic dextrose

consistently induced fibrosis in a larger series of animals, and to determine if such changes were associated with changes in nerve conduction and SSCT material properties, similar to those seen in patients with CTS.

METHODS

Surgical Procedure

The experimental protocol was reviewed and approved by our institutional animal care and use committee. Twenty-eight New Zealand white rabbits weighing 4.0 to 4.5kg were used. The rabbits were anesthetized by an intramuscular injection of ketamine hydrochloride (50mg/kg) and xylazine (10mg/kg). Following the induction of anesthesia, both forepaws were prepared and draped. One fore paw was randomly selected for injection of 0.1ml of 10% dextrose solution^a into the SSCT under direct vision. The rabbit carpal tunnel was identified by locating a small but easily palpable nodule of subcutaneous cartilage which, in the rabbit, forms part of the transverse carpal ligament. Using this landmark to locate the carpal tunnel, a 3.0 to 4.0 mm incision was made in the paw 1cm proximal to the carpal tunnel. The flexor tendons were identified and the middle digit FDS tendon was identified by moving that digit passively. A 30 gauge needle was inserted into along the surface of the middle digit FDS to ensure that the injection was made into the synovium between the middle digit FDS tendon and the transverse carpal ligament. The distal extent of needle penetration was confirmed by placing the same size needle on the skin surface. The contralateral paw was injected with a similar amount of 0.9% saline solution as a control. The wounds were closed with sutures of 5-0 Vicryl^b. On awakening, the rabbits were allowed full cage activity. After 12 weeks of observation, the rabbits were prepared for the following evaluations.

Electrophysiologic Analysis

EP testing was performed on the median nerve of each forepaw in 12 animals under general anesthesia, administered as described above. The CMAP was recorded from the thenar muscle with stimulation of the median nerve 3cm proximal to the recording point. Stimulation was carried out until a supramaximal response was visualized on the monitor. Recording was performed before injection and 12 weeks after injection, immediately prior to sacrifice. We also measured CMAP latency to onset and amplitude.

Mechanical Testing

After sacrificing the animals, the fore paws of 8 animals were harvested for mechanical testing with the carpal tunnel intact. The method has been described previously.²⁷ In brief, the FDS tendons were exposed at the antebrachial level, the middle digit FDS tendon was divided at a level 5mm proximal to the proximal edge of the flexor retinaculum, and the proximal end of the middle digit FDS tendon was sutured with 5-0 Vicryl as an anchor cable. The specimen was then mounted in a custom fixture. The fixture was mounted on a custom-made microtester, which was composed of a linear servo motor^c and a load cell with the accuracy of 0.01N^d. The anchor cable was connected to the load cell. The middle digit FDS tendon was then sharply cut 5 mm distal to the distal edge of the carpal tunnel.

Under displacement control, the middle digit FDS tendon was moved through the carpal tunnel at a rate of 0.5mm/s. The load cell measurements and displacement were recorded through a LabVIEW program^e. Throughout testing, the specimen was kept moist by spraying saline

^aHospira Inc, 275 N Field Dr, Lake Forest, IL 60045.

^bEthicon, Route 22 West, Somerville, NJ 08876.

^cMX 80 Daedal; Daedal, 1140 Sandy Hill Rd, Irwin, PA 15642.

^dMDB-5; Transducer Techniques, 42480 Rio Nedo, Temecula, CA 92590.

solution. The ultimate tensile load, the energy absorption and the stiffness of the SSCT surrounding the middle digit FDS tendons were analyzed (fig 1). The ultimate tensile load and energy absorption were defined as the maximum load and the area under the load/displacement curve, respectively. The stiffness was defined as the slope of the load/displacement curve. For the stiffness measurement, the displacement at the ultimate tensile load was defined as 100% (the ultimate load displacement). The stiffness was then measured for each 10% increment of displacement until the ultimate load displacement.

Histologic Analysis

After sacrificing the animals, the fore paws of 8 animals were harvested for histology. After removing the skin, the fore paw was placed in 4% paraformaldehyde solution for 24 hours for primary fixation. Then, the tissue was divided into 2 groups, to analyze the subsynovial connective tissue and median nerve. For the subsynovial connective tissue evaluation, the carpal tunnel tissue was immersed into formalin and embedded in paraffin. Five micron cross sections were made through the carpal tunnel and stained with standard hematoxylin and eosin. For the median nerve evaluation, the tissue was immersed in a fixative solution of 10% glutaraldehyde and 10% paraformaldehyde. The tissue was embedded in plastic resin. 0.6µm cross sections of the median nerve were made at the carpal tunnel level and stained with toluidine blue. Sections were evaluated by qualitative assessment under a light microscope^f. For the SSCT assessments, the SSCT thickness was assessed at low magnification (20×), while fibrosis, cellularity, and neovascularization were assessed at high magnification (400×). For the median nerve assessments, fascicular demyelination and subperineurial edema were observed for three different fascicles at high magnification (400×, 1000×).

Statistical Analysis

For the EP tests, a 2-factor analysis of variance with repeated measures was used to evaluate the effects of observation period and injection, and the interaction between these 2 factors. These factors were also analyzed by *t* test. Mechanical data had only 1 factor (treatment) to assess. These data were analyzed with the paired *t*-test. All analyses were performed by SAS/STAT version 9.1.3 software^g. The results were expressed as mean ± SD. *P* values less than .05 were considered statistically significant.

RESULTS

Electrophysiologic Analysis

Summary results of electrophysiologic analysis are presented in table 1. There was no significant difference when looking at the interaction of the observation period and injection for either amplitude (*P*=.11) or latency (*P*=.07). By *t* test, the distal motor latency did show a significant delay at 12 weeks in the dextrose group compared to the saline group (*P*<.05).

Mechanical Property Tests

The mean ultimate tensile loads were 960.4±479.7mN in the dextrose group and 724.3±322.5mN in the saline group. These results were not statistically different (fig 2A). The mean energy absorptions were 6.23±3.31mJ in the dextrose group and 4.09±1.98mJ in the saline group. There was a significant difference in energy absorption (fig 2B) (*P*<.05). The stiffness of the SSCT also showed a significant difference at 50% to 60% and 90% to 100% displacement (fig 3A) (*P*<.05).

^eNational Instruments, 11500 N MoPac Expwy, Austin, TX 78759-3504.

^fBLX51; Olympus Co, Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-0914.

^gSAS Institute Inc, 100 SAS Campus Dr, Cary, NC 27513-2414.

Histologic Analysis

The SSCT consisted of collagen bundles which were connected to 1 another by smaller bundles. In the dextrose group, the collagen bundles were thicker than in the saline group (fig 4). In addition, the dextrose specimens showed hypercellularity and vascular proliferation within the SSCT (fig 5A) when compared to saline specimens (fig 5B). The nerve histology was not obviously different when comparing the dextrose and saline specimens (fig 6A, B).

DISCUSSION

This study assessed the biologic effects of hypertonic dextrose injection on rabbit carpal tunnel SSCT. We demonstrated that a single injection of 10% dextrose induced SSCT fibrosis, and also resulted in focal slowing of median nerve motor conduction velocity and decreased motor amplitude. In addition, the SSCT material properties changed, with increased energy absorption and stiffness.

Prolotherapy is an injection-based treatment for chronic musculoskeletal pain. Its proposed mode of action is through the strengthening of stretched or torn connective tissue.²⁸ Although the mechanism of this treatment is not fully understood, animal biopsy studies show ligament thickening, enlargement of the bone-tendon junction, and strengthening of the tendon or ligament after prolotherapy injection.^{23,29} In this study, we chose 10% dextrose as the stimulant. Dextrose concentrations above 10% lead to inflammatory cascade activation, in part by an osmotic stress effect.^{30–32} However, 10% dextrose, with an osmolarity of 505mOsm, is below the level at which cell crenation occurs, and thus is not enough to activate the inflammatory cascade.³³ Because the purpose of this study was to investigate the effect of noninflammatory fibrosis, this dextrose concentration is appropriate to consider.

The action of noninflammatory dextrose concentrations on fibroblasts is profound, even at 0.6%, and has been studied in human and animal cells in vitro extensively. It exerts a strong influence on proliferation of cells such as chondrocytes, osteocytes, and fibroblasts.³⁴ It also influences protein synthesis and amino acid transport without any cellular toxicity,³⁵ and produces less pain than higher concentration (20%) of dextrose solution does.²⁴ Liu et al³⁶ showed that extracellular matrix components of fibrosis (collagen type I and fibronectin) were produced in human vascular smooth muscles cells within hours of exposure to high glucose concentration and that this occurred via upregulation of CTGF. A further example of the complexity of the effect of noninflammatory glucose elevations are that production of collagen 1 and 3 require the presence of IGF along with CTGF in human renal fibroblasts.³⁷

The effect of 10% dextrose is not based on osmolarity alone. A stress of 1100mOsm saline was not nearly as potent in stimulating regeneration within rat Achilles tendon as was 5% dextrose made 1100mOsm by mixing with hypertonic saline.³⁸ This clearly indicates an effect of dextrose that is not just osmotic. Also pertinent is a study showing that nonsteroidal anti-inflammatory drug administration did not eliminate the effect of 20% dextrose on fibroblast proliferation in the rat Achilles tendon.³⁹

We have shown that a single injection of 10% dextrose, which is metabolized and removed within hours by the local cells, stimulated proliferation of collagen over time (12 weeks) in both this study and a pilot study.²⁶ These changes are similar to the changes seen in the SSCT in CTS patients, including a marked increase in fibroblast density, collagen fiber size,¹¹ deformation of collagen fibrils and phagocytosis of aberrant collagen fibrils and elastin.⁴⁰ Although the mechanism of action of hypertonic dextrose on the SSCT remains unclear with our current data, we believe that we have sufficient evidence of an effect now to initiate further, mechanistic investigations, based on the protocol we confirmed here.

In addition to confirming the findings of the pilot study, we have now shown that EP changes can be observed after hypertonic dextrose injection into the SSCT, which are also similar to those seen in patients with CTS. It is important to note that the most sensitive EP findings in CTS are those of sensory amplitude and latency.^{41,42} Motor nerve changes, such as we observed, are a relatively late finding. Unfortunately, it is technically quite challenging to perform median sensory nerve conduction studies in the rabbit model. Thus we were gratified to observe significant changes in what is, clinically, a less sensitive measure of median nerve function. With a larger sample size, the amplitude changes may also have become significant. However, our power calculation was based on discerning relatively large changes, as would be seen clinically in patients with advanced carpal tunnel syndrome.

The mechanical properties of the SSCT showed marked changes after dextrose injection, as compared to the saline injected paws. The ultimate tensile load, energy absorption, and stiffness all increased after hypertonic dextrose injection. The increases in these indices suggest that the effect of the injection has been to tether the SSCT more tightly to the middle digit FDS tendon. This is also similar to observation in CTS patients.^{43,44} Osamura et al⁴⁵ investigated the material properties of the SSCT in response to shear stress in human specimens. The shear force of the SSCT in CTS patients was significantly higher than that of the healthy specimens, again a finding we observed in our dextrose treated paws.

Taken together, we believe that our findings suggest that the SSCT changes seen with dextrose injection parallel those seen in CTS patients. Consistent with this observation, we also noted early changes in motor nerve function. While the nerve histology was not obviously different when comparing the dextrose and saline groups, we did not undertake morphometric analysis of nerve nor undertake single teased fiber evaluation for demyelination, so at this point we can not draw any further conclusions regarding the effect of the dextrose injection on nerve pathology. Of course, as nerve biopsies are never done in patients with CTS, we do not know what would be observed in patients who presented with similar changes in motor nerve conduction.

Study Limitations

There are several limitations to this study. Because we evaluated a single time point, we have no data on the time course of the changes we observed. A pilot study showed sequential structural changes in the SSCT and median nerve in a rabbit model.²⁶ Because we wanted to evaluate evidence of demyelination, we used a different embedding process for nerve histology than had been used in the pilot study. Thus, it is difficult to compare the nerve histology results in the 2 studies. The increase in latency should reflect paranodal demyelination, an alteration that might not be apparent on cross-sectional evaluation. In the future, single teased fiber evaluation will be necessary to evaluate paranodal changes. Our decision to test a single time point was based on a desire to confirm the pilot data, and extend the findings to EP, before embarking on a much larger study, with more animals. Also, we did not quantify the thickening of SSCT in this study. We plan to develop a method to evaluate the thickness and volume of SSCT in a future study, perhaps using high resolution magnetic resonance imaging. Based on our results here, we will now look at SSCT changes and EP both at times before and after 12 weeks. In addition, we will use this model to look at dose-effect relationships and interventions that are not possible either in vitro or clinically. This information will be useful to better quantify the phenomena we observed here. In addition to providing an opportunity to investigating the relationship between SSCT changes and median nerve EP, these studies may also help us to understand the effect of prolotherapy on enthesopathies.

CONCLUSIONS

This study is unique among carpal tunnel models in its specific focus on early changes and the role of the connective tissue, similar to what is presumed to occur with chronic repetitive stress in early carpal tunnel syndrome. We assessed the biologic effect of hypertonic dextrose injection on the rabbit carpal tunnel SSCT. We demonstrated that injection of 10% dextrose induced a delay of median nerve conduction, SSCT mechanical property changes and fibrosis. These are consistent with the findings in CTS patients. These results suggest that hypertonic dextrose injection has a potential to create a novel animal model in which to study the evolution of CTS, and provide the groundwork on which to build future studies. We are currently planning to evaluate the electrophysiologic alterations of amplitude and latency associated with repetitive injections, and if these are associated with more florid changes in peripheral nerve, including demyelination and axonal degeneration.

Acknowledgments

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List of Abbreviations

CMAP	compound muscle action potential
CTGF	connective tissue growth factor
CTS	carpal tunnel syndrome
EP	electrophysiology
FDS	flexor digitorum superficialis
SSCT	subsynovial connective tissue

References

1. Atroshi I, Gummesson C, Johnsson R, Ornstein E, Ranstam J, Rosén I. Prevalence of carpal tunnel syndrome in a general population. *JAMA* 1999;282:153–8. [PubMed: 10411196]
2. de Krom MC, Knipschild PG, Kester AD, Thijs CT, Boekkooi PF, Spaans F. Carpal tunnel syndrome: prevalence in the general population. *J Clin Epidemiol* 1992;45:373–6. [PubMed: 1569433]
3. Bland JD, Rudolfer SM. Clinical surveillance of carpal tunnel syndrome in two areas of the United Kingdom, 1991–2001. *J Neurol Neurosurg Psychiatry* 2003;74:1674–9. [PubMed: 14638888]
4. Bongers FJ, Schellevis FG, van den Bosch WJ, van der Zee J. Carpal tunnel syndrome in general practice (1987 and 2001): incidence and the role of occupational and non-occupational factors. *Br J Gen Pract* 2007;57:36–9. [PubMed: 17244422]
5. Stevens JC, Sun S, Beard CM, O'Fallon WM, Kurland LT. Carpal tunnel syndrome in Rochester, Minnesota, 1961 to 1980. *Neurology* 1988;38:134–8. [PubMed: 3336444]
6. Nordstrom DL, DeStefano F, Vierkant RA, Layde PM. Incidence of diagnosed carpal tunnel syndrome in a general population. *Epidemiology* 1998;9:342–5. [PubMed: 9583428]

7. Gelberman RH, Hergenroeder PT, Hargens AR, Lundborg GN, Akeson WH. The carpal tunnel syndrome. A study of carpal canal pressures. *J Bone Joint Surg Am* 1981;63:380–3. [PubMed: 7204435]
8. Szabo RM, Chidgey LK. Stress carpal tunnel pressures in patients with carpal tunnel syndrome and normal patients. *J Hand Surg Am* 1989;14:624–7. [PubMed: 2754193]
9. Werner CO, Elmqvist D, Ohlin P. Pressure and nerve lesion in the carpal tunnel. *Acta Orthop Scand* 1983;54:312–6. [PubMed: 6846011]
10. Guimberteau, JC. New ideas in hand flexor tendon surgery. XX: Institut Aquitain De La Main; 2001. The sliding system. Vascularized flexor tendon transfers.
11. Ettema AM, Amadio PC, Zhao C, Wold LE, An KN. A histological and immunohistochemical study of the subsynovial connective tissue in idiopathic carpal tunnel syndrome. *J Bone Joint Surg Am* 2004;86:1458–66. [PubMed: 15252093]
12. Oh J, Zhao C, Amadio PC, An KN, Zobitz ME, Wold LE. Vascular pathologic changes in the flexor tenosynovium (subsynovial connective tissue) in idiopathic carpal tunnel syndrome. *J Orthop Res* 2004;22:1310–5. [PubMed: 15475214]
13. Fuchs PC, Nathan PA, Myers LD. Synovial histology in carpal tunnel syndrome. *J Hand Surg Am* 1991;16:753–8. [PubMed: 1880380]
14. Neal NC, McManners J, Stirling GA. Pathology of the flexor tendon sheath in the spontaneous carpal tunnel syndrome. *J Hand Surg Br* 1987;12:229–32. [PubMed: 3624984]
15. Luch AL. Thickening of the synovium of the digital flexor tendons: cause or consequence of the carpal tunnel syndrome? *J Hand Surg Br* 1992;17:209–12. [PubMed: 1588206]
16. Lim JY, Cho SH, Han TR, Paik NJ. Dose-responsiveness of electrophysiologic change in a new model of acute carpal tunnel syndrome. *Clin Orthop Relat Res* 2004;(427):120–6. [PubMed: 15552147]
17. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Chronic nerve compression--an experimental model in the rat. *Ann Plast Surg* 1984;13:112–20. [PubMed: 6476732]
18. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. A primate model for chronic nerve compression. *J Reconstr Microsurg* 1985;1:185–95. [PubMed: 4057158]
19. Hackett, GS.; Hemwall, GA.; Montgomery, GA. Ligament and tendon relaxation treated by prolotherapy. Vol. 3. Springfield: Charles C Thomas; 1958.
20. Topol GA, Reeves KD, Hassanein KM. Efficacy of dextrose prolotherapy in elite male kicking-sport athletes with chronic groin pain. *Arch Phys Med Rehabil* 2005;86:697–702. [PubMed: 15827920]
21. Reeves, KD. Pain procedures in clinical practice. Vol. 2. Philadelphia: Hanley and Belfus; 2000. Prolotherapy: basic science, clinical studies and technique; p. 172-90.
22. Alderman D. Prolotherapy for musculoskeletal pain. *Practical pain management* 2007;7:10–6.
23. Maynard JA, Pedrini VA, Pedrini-Mille A, Romanus B, Ohlerting F. Morphological and biochemical effects of sodium morrhuate on tendons. *J Orthop Res* 1985;3:236–48. [PubMed: 3998899]
24. Reeves KD, Hassanein KM. Long-term effects of dextrose prolotherapy for anterior cruciate ligament laxity. *Altern Ther Health Med* 2003;9:58–62. [PubMed: 12776476]
25. Park YS, Lim SW, Lee IH, Lee TJ, Kim JS, Han JS. Intra-articular injection of a nutritive mixture solution protects articular cartilage from osteoarthritic progression induced by anterior cruciate ligament transection in mature rabbits: a randomized controlled trial. *Arthritis Res Ther* 2007;9:R8. [PubMed: 17257416]
26. Oh S, Ettema AM, Zhao C, et al. Dextrose induced subsynovial connective tissue fibrosis in rabbit carpal tunnel. *Hand* 2008;3:34–40. [PubMed: 18780118]
27. Yamaguchi T, Osamura N, Zhao C, Zobitz ME, An KN, Amadio PC. The mechanical properties of the rabbit carpal tunnel subsynovial connective tissue. *J Biomech*. 2007Epub ahead of print
28. Klein RG, Dorman TA, Johnson CE. Proliferant injections for low back pain: histologic changes of injected ligaments and objective measurements of lumbar spine mobility before and after treatment. *J Neuro Orthop Med Surg* 1989;10:123–6.
29. Liu YK, Tipton CM, Matthes RD, Bedford TG, Maynard JA, Walmer HC. An in situ study of the influence of a sclerosing solution in rabbit medial collateral ligaments and its junction strength. *Connect Tissue Res* 1983;11:95–102. [PubMed: 6224646]

30. Jensen KT, Rabago DP, Best TM, Patterson JJ, Vanderby RJ. Early inflammatory response of knee ligaments to prolotherapy in a rat model. *J Orthop Res* 2008;26:816–23. [PubMed: 18240327]
31. Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996;45:954–9. [PubMed: 8666148]
32. Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* 2003;52:1256–64. [PubMed: 12716761]
33. Calder PC, Dimitriadis G, Newsholme P. Glucose metabolism in lymphoid and inflammatory cells and tissues. *Curr Opin Clin Nutr Metab Care* 2007;10:531–40. [PubMed: 17563475]
34. Reeves KD, Hassanein K. Randomized prospective double-blind placebo-controlled study of dextrose prolotherapy for knee osteoarthritis with or without ACL laxity. *Altern Ther Health Med* 2000;6:68–74. [PubMed: 10710805]
35. Borghetti P, Della Salda L, De Angelis E, et al. Adaptive cellular response to osmotic stress in pig articular chondrocytes. *Tissue Cell* 1995;27:173–83. [PubMed: 7778094]
36. Liu X, Luo F, Pan K, Wu W, Chen H. High glucose upregulates connective tissue growth factor expression in human vascular smooth muscle cells. *BMC Cell Biol* 2007;8:1. [PubMed: 17224075]
37. Lam S, van der Geest RN, Verhagen NA, et al. Connective tissue growth factor and igf-I are produced by human renal fibroblasts and cooperate in the induction of collagen production by high glucose. *Diabetes* 2003;52:2975–83. [PubMed: 14633859]
38. Kim HJ, Jeong TS, Kim WS, Park YS. Comparison of histological changes in accordance with the level of dextrose-concentration in experimental prolotherapy model. *J Korean Acad Rehabil Med* 2003;27:935–40.
39. Kim HJ, Kim SH, Yun DH, Lee KS, Jeong TS. The effects of anti-inflammatory drugs on histologic findings of the experimental prolotherapy model. *J Korean Acad Rehabil Med* 2006;30:378–84.
40. Oh J, Zhao C, Zobitz ME, Wold LE, An KN, Amadio PC. Morphological changes of collagen fibrils in the subsynovial connective tissue in carpal tunnel syndrome. *J Bone Joint Surg Am* 2006;88:824–31. [PubMed: 16595473]
41. Atroshi I, Gummesson C, Johnsson R, Ornstein E. Diagnostic properties of nerve conduction tests in population-based carpal tunnel syndrome. *BMC Musculoskelet Disord* 2003;4:9. [PubMed: 12734018]
42. Monga TN, Shanks GL, Poole BJ. Sensory palmar stimulation in the diagnosis of carpal tunnel syndrome. *Arch Phys Med Rehabil* 1985;66:598–600. [PubMed: 4038024]
43. Ettema AM, Zhao C, Amadio PC, O’Byrne MM, An KN. Gliding characteristics of flexor tendon and tenosynovium in carpal tunnel syndrome: a pilot study. *Clin Anat* 2007;20:292–9. [PubMed: 16944527]
44. Nakamichi K, Tachibana S. Restricted motion of the median nerve in carpal tunnel syndrome. *J Hand Surg Br* 1995;20:460–4. [PubMed: 7594983]
45. Osamura N, Zhao C, Zobitz ME, An KN, Amadio PC. Evaluation of the material properties of the subsynovial connective tissue in carpal tunnel syndrome. *Clin Biomech* 2007;22:999–1003.

Fig 1.

Typical force curve and indices used for the mechanical property assessment. (A) Ultimate tensile load. (B) Stiffness; the displacement at the ultimate tensile load was defined as 100% (the ultimate load displacement). The stiffness was defined by the tilt of each 10% displacement until the ultimate load displacement. *Energy absorption; the area under the shear force curve.

Fig 2. Mechanical property results. Error bar indicates 1 SD. (A) Ultimate tensile load. (B) Energy absorption. * $P < .05$.

Fig 3.

Mechanical property results. Error bars indicate 1 SD. (C) Stiffness, with total excursion broken into 10% increments. Black bars: dextrose, white bars: saline. * $P<.05$. (D) Representative curve. Dark line: dextrose, light line: saline.

Fig 4.

Example of SSCT histology results (HE, 20 $\times$). (A) Dextrose group. (B) Saline group. Profundus tendon, arrow: SSCT. Arrows delineate the thickness of SSCT. Scale bar indicates 1.0mm. Abbreviations: Fd, flexor digitorum; Fs, flexor digitorum superficialis tendon; N, median nerve.

Fig 5.
Example of SSCT histology results (HE, 400×). (A) Dextrose group. (B) Saline group. Scale bar indicates .05mm.

Fig 6. Example of Nerve Histology Results (Toluidine blue, 400 $\times$). (A) Dextrose group. (B) Saline group. Scale bar indicates .05mm.

Table 1

Results of EP Testing

	Dextrose	Saline
Latency (ms)		
Initial	1.74±0.25	1.73±0.16
12 weeks after injection	1.92±0.41	1.60±0.21
Amplitude (mV)		
Initial	1.63±0.60	1.79±0.66
12 weeks after injection	1.23±0.60	1.66±0.61

NOTE. Values are mean ± SD.

*

There was a significant difference between dextrose and saline group after 12 weeks of injection ($P<.05$).